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Coordinative task difficulty and behavioural errors are associated with increased long-range beta band synchronization

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Abstract

The neural network and the task-dependence of (local) activity changes involved in bimanual coordination are well documented. However, much less is known about the functional connectivity within this neural network and its modulation according to manipulations of task complexity. Here, we assessed neural activity via high-density electroencephalography, focussing on changes of activity in the beta frequency band (~15-30 Hz) across the motor network in 26 young adult participants (19-29 years old). We investigated how network connectivity was modulated with task difficulty and errors of performance during a bimanual visuomotor movement consisting of dial rotation according to three different ratios of speed: an isofrequency movement (1:1), a non-isofrequency movement with the right hand keeping the fast pace (1:3), and the converse ratio with the left hand keeping the fast pace (3:1). To quantify functional coupling, we determined neural synchronization which might be key for the timing of the activity within brain regions during task execution. Individual source activity with realistic head models was reconstructed at seven regions of interest including frontal and parietal areas, among which we estimated phase-based connectivity. Partial least squares analysis revealed a significant modulation of connectivity with task difficulty, and significant correlations between connectivity and errors in performance, in particular between sensorimotor cortices. Our findings suggest that modulation of long-range synchronization is instrumental for coping with increasing task demands in bimanual coordination.

Keywords

Bimanual coordination, visuomotor integration, beta band synchronization, partial least squares (PLS), exact low-resolution tomography analysis (eLORETA), finite element modelling (FEM).

Introduction

Task difficulty in bimanual coordination depends on the spatiotemporal characteristics of the submovements and overall movement speed. Simultaneous (in-phase) or alternated movement (anti-phase) of the limbs are the easiest bimanual coordination patterns that belong to the intrinsic motor repertoire, and deviations from these require more attention and practice (Kelso, 1984; Zanone & Kelso, 1992; Lee *et al.*, 1995; Treffner & Turvey, 1995; Serrien & Swinnen, 1997). There are two principal ways in which the central nervous system copes with the task demands: via increased neural activation (either within the same region or recruiting additional regions) and via modulations of the strength of interaction among those regions, i.e. functional connectivity. The latter has received little attention, in spite of evidence showing that modulated communication among brain regions is key for successful multi-sensory integration or execution of cognitive tasks (e.g. Roelfsema *et al.*, 1997; Maier *et al.*, 2008; Pesaran *et al.*, 2008).

Increased neural activation in bimanual movements compared to rest has been reported in what is referred to as the bimanual neural network. Among other regions, this network comprises the primary motor cortex (M1), supplementary motor area (SMA), premotor cortex (PMC), cingulate motor area, basal ganglia and cerebellum (Swinnen & Wenderoth, 2004; Pollok *et al.*, 2007). A higher movement frequency during stable execution involves activation in the bilateral SMAs, premotor dorsal (PMd) and left middle cingulate cortex (Sadato *et al.*, 1996; Deiber *et al.*, 1999; Debaere *et al.*, 2004; Goble *et al.*, 2010) and a linear decrease of power in the beta frequency band (approx. 13-30 Hz) in bilateral M1s (Houweling *et al.*, 2010). It should be noted that decreases of power in the alpha (8-12 Hz) and beta bands in electrophysiological measures are considered markers of neural activation in motor-related tasks (Pfurtscheller & Neuper, 1994; Stancák Jr & Pfurtscheller, 1996).

Task difficulty manipulated via phase relations revealed that anti-phase movements are accompanied by increased activity in the SMA and PMC compared to in-phase (Sadato *et al.*, 1997; Goerres *et al.*, 1998; Stephan *et al.*, 1999; Immisch *et al.*, 2001; Gross *et al.*, 2005; Aramaki *et al.*, 2006; Pollok *et al.*, 2007), as well as in the brain stem, cerebellum, and M1 (Aramaki *et al.*, 2006). Besides activity increases, EEG activity during in-phase and anti-phase movements results from temporal modulations of unimanual-related EEG activity (Banerjee *et al.*, 2012). Phase relations that differ from anti-phase and in-phase come with activity increases in the SMA, superior parietal cortex, thalamus, cerebellum and PMd (Debaere *et al.*, 2003; Debaere *et al.*, 2004). Similarly, bimanual movements whereby each hand moves at a different frequency, i.e. non-isofrequency rhythms or polyrhythms, are associated with activity increases in bilateral M1s and medial brain regions (Lang *et al.*, 1990; Ullén *et al.*, 2003).

Besides increases of local activation as a function of task difficulty, alterations in functional connectivity may be an additional means to cope with task difficulty. Inter-hemispheric connectivity between bilateral primary sensorimotor cortices (S1/M1) in the beta frequency band has been reported when increasing the task demands from unimanual, to bimanual in-

phase and anti-phase, mostly at the sensor level, in magneto- and electro-encephalography (M/EEG) studies (Andres *et al.*, 1999; Serrien & Brown, 2002; Serrien *et al.*, 2003; Serrien, 2008b; 2009; Houweling *et al.*, 2010). Higher inter-hemispheric connectivity was also observed in bimanual compared to unimanual movements or rest in studies using functional magnetic resonance imaging (fMRI) (Rissman *et al.*, 2004; Grefkes *et al.*, 2008; Maki *et al.*, 2008). However there are dissenting findings. Walsh *et al.* (2008) did not find any changes in inter-hemispheric connectivity between bilateral M1s, but found bilateral SMA connectivity increases. Also, Serrien (2008a) reported decreased inter-hemispheric connectivity in the beta band during the in-phase coordination mode compared to the anti-phase and unimanual ones.

With this study, we addressed three gaps in the literature. First, studies investigating modulations of brain activity associated with difficulty of bimanual tasks beyond the "simple" in-phase/anti-phase coordination modes have been limited to manipulating either the overall speed of movement (Houweling *et al.*, 2010) or the phase relations (Debaere *et al.*, 2004). Modulations of the bimanual neural network as a function of *manipulation of the spatiotemporal features* of bimanual movements (e.g. different non-isofrequency movements), implying different task assignments to each limb, have to date only been investigated with fMRI (Ullén *et al.*, 2003). Compared to the preferred in- and anti-phase movements, non-isofrequency movements require preservation of the allocated pace in each hand within an integrated temporal framework across both hands. This is based on modulation of inter-hemispheric interactions between premotor and primary motor cortices to gate the different task assignments to each limb in contrast to isofrequency conditions where this is not the case (Fujiyama *et al.*, 2016a; Fujiyama *et al.*, 2016b). Second, functional connectivity changes have been studied primarily between bilateral S1/M1s and SMA, with no focus on the involvement of a *broader network*. There are, however, a few exceptions to this. In an fMRI study, Grefkes *et al.* (2008) reported increased inter-and intra-hemispheric connectivity during bimanual movements compared to unimanual movements in a network including bilateral S1/M1s, PMCs and SMA. Using fMRI, Heitger *et al.* (2012; 2013) demonstrated increases of functional connectivity, with motor learning and in anti-phase compared to in-phase bimanual movements using the bimanual network extracted from activity profiles (i.e. a data-driven approach) including distant brain regions. Third, connectivity analysis at the *source level using M/EEG* has not been applied before to investigate modulations related to bimanual task difficulty. The local and long-range synchronization of neural dynamics as measured via M/EEG can be considered an information carrier between neural populations (Lopes da Silva, 2013). However most EEG literature on bimanual coordination uses sensor-level connectivity measures which can produce spurious patterns (Sakkalis, 2011).

Here we sought to address these gaps by applying a multivariate method to characterise the patterns of functional connectivity of source-level EEG and assess their modulations with task difficulty defined by different spatiotemporal characteristics. Additionally, given the limited literature on the topic, we also examined the underlying neural mechanisms of adaptation to task demands by investigating correlations of connectivity with behaviour. We first recorded high-density EEG during the execution of a bimanual visuomotor task, which consisted of

circular wrist movements at different speeds for each hand. We estimated source activity using MRI-based individual head models, determined the synchronization level among the sources, and applied partial least squares analysis to examine network modulations with task difficulty and correlations of network modulations with error of performance (Boonstra *et al.*, 2007; McIntosh *et al.*, 2014). At the behavioural level, we expected an increase of performance error with increasing task difficulty. At the source level, we sought to reproduce the aforementioned motor network for bimanual motor behaviour, and hypothesised increases of activity in bilateral S1/M1s as a function of task difficulty. At the network level, we further hypothesised an increase of connectivity with increased task difficulty as this may support coping with the elevated task demands. Additionally, we expected correlations of connectivity with performance error to confirm network modulations with task difficulty.

Methods

Participants

Twenty-six participants were recruited and provided informed consent for this experiment (11 male; mean age 24.17 years; range 19-29 years) which had been approved by the ethics committee of KU Leuven. All participants were right-handed according to the Oldfield Handedness Questionnaire (mean 91.09; range: 56-100) (Oldfield, 1971).

Setup and task

Participants practiced a bimanual visuomotor task similar to the one previously used in our laboratory (Sisti *et al.*, 2011; Gooijers *et al.*, 2013; Pauwels *et al.*, 2014; Solesio-Jofre *et al.*, 2014; Beets *et al.*, 2015). Participants were seated in front of a computer while their arms rested on two ramps covered with foam for comfort. At the end of each ramp, a shaft embedded into a rotating disc was placed. The rotating disc was glued to an encoder for registration of angular displacement (Avago Technologies, Fs= 250 Hz, accuracy= 0.089°). Participants were instructed to hold the dials as if they were pens. In order to prevent participants from seeing their hands/forearms, a wooden frame was positioned on top of their limbs. The data from the encoders was recorded and analysed with LabView 8.5 (National Instruments, Austin, Texas, USA).

The rotation of the dials, one for each hand, yielded a movement of a red cursor on the PC screen. Clockwise rotation of the right dial moved the cursor to the right; counter-clockwise rotation moved the cursor to the left (see Fig. 1). Clockwise rotation of the left dial moved the cursor upwards; counter-clockwise rotation moved the cursor downwards. Simultaneous rotation of both dials resulted in lines with different slopes being drawn on the PC screen, depending on the rotational speed of both hands. A blue line with a particular slope was shown to provide a cue (target) for the required coordination pattern. There were three task conditions of varying frequency ratios as follows: a) the isofrequency movement or 1:1 ratio whereby a line on the diagonal (with a slope of 45° with respect to the horizontal axis) indicated a synchronous/symmetric movement of the hands; b) the 1:3 ratio whereby a line

closer to the horizontal axis indicated a non-isofrequency movement in which the right hand had to move *3* times faster than the left hand; and c) the 3:1 ratio whereby a line closer to the vertical axis indicated the mirrored rhythm, in which the left hand had to move *3* times faster than the right hand. Additionally, a white dot moved along the target line indicating the tempo at which the movement should be undertaken. Online feedback of the movement was provided throughout the trials. The goal of the task was to rotate the dials such that the line drawn by the participant would match the cue line as close as possible at the tempo indicated by the moving white dot.

A trial started with the line slope shown on the screen. For 2 s, a yellow circle was shown in the starting position indicating that no movement was required from the participants. Once the yellow circle disappeared, the white dot started moving along the line and the participants were required to move the dials. The execution of the movement lasted 8 s and the inter-trial interval was 4 s. To reduce movement artefacts in the EEG recordings, the first and last second of the trial were rejected (see below in EEG pre-processing).

Experimental design

The experiment consisted of four consecutive phases. First, participants looked at a white cross on the screen for 3 min to record the resting state with EEG. Second, we explained the task to the participants and they practiced the task for 25 min to familiarise themselves with the task and equipment. The three conditions (1:1, 1:3 and 3:1) were presented randomly in blocks of 15 trials. Third, the participants performed the task for 40 min while EEG was recorded. Participants were instructed to perform the task as gently as possible to minimise muscle artefacts in the EEG. Whenever needed, participants took breaks between blocks.

EEG acquisition

EEG was recorded with a high-density 128-channel amplifier of ANT (Enschede, Netherlands; sampling frequency Fs= 1024 Hz) with average reference. Eye movement was recorded with 2 bipolar EOG channels. The location of electrodes was digitised and recorded with an infrared camera (Visor2, ANT).

MRI acquisition

For creating the individual head models, we obtained the structural MRI scans of all participants. A 3D high-resolution T1-weighted image was obtained using a Siemens 3-T Magnetom Trio MRI scanner (Siemens, Erlangen, Germany) with a 12-channel head coil. A magnetisation prepared rapid gradient echo (MPRAGE) sequence lasting 8 min was used (time repetition/time echo = 2300/ 2.98 ms, $1 \times 1 \times 1.1$ mm^3 voxels, field of view (FOV) = 240×256, 160 sagittal slices).

Kinematics analysis and statistics

The *x* and *y* positions of the target and participant's cursor on the screen served to determine two performance measures: the participant's cursor-target deviation and the frequency-ratio deviation. The cursor-target deviation consisted of the Euclidean distance between the target and the participant's cursor measured in arbitrary (but fixed) units. This measure provides a global metric of the performance of the visuomotor task, including the tempo, the movement pattern and the position of the cursor with respect to the moving target (see Fig. S1.a). The frequency-ratio deviation consisted of the difference in degrees between the angle of the target line and the angle of the line drawn by the participant. This describes how well the coordination pattern is produced regardless of the tempo or the position of the participant's line on the screen (see Fig. S1.b). Both performance measures were correlated with network modulations to test for the performance of the visuomotor task and the movement pattern per se (see Phase-based connectivity for details). Both measures were averaged across time-points during execution and across trials per condition per participant.

Two one-way ANOVAs with repeated measures were used to evaluate the effect of the conditions on these measures, with frequency ratio as the single factor with three levels (1:1, 1:3 and 3:1). The data was log-transformed to comply with the normality requirement. We performed post-hoc (paired) t-tests to evaluate the differences between pairs of conditions. For all hypothesis testing the significance level was set to α=0.05. This analysis was conducted in Matlab R2011b (The Mathworks) and SPSS 22 (IBM).

Electroencephalography

Pre-processing

Resting-state and task-related EEG recordings were band-pass filtered at 1-100 Hz and down-sampled to 256 Hz. To minimise artefacts, four steps were conducted. First, the initial and last seconds of the execution interval of the trial were rejected, as they were noisy in most participants due to initial adjustments at the beginning and brisk movements in the end. That left epochs of 6 s, which were redefined as 2-s segments of these 6-s intervals. The epochs for the resting-state EEG were also redefined as 2-s segments. Second, epochs with voltage levels exceeding 150 µV were rejected. Third, independent component analysis (ICA) was applied to extract components related to eye blinks and movement artefacts. Components related to eye blinks/movements were selected based on the highest weights of the mixing matrix contributing to EOG channels. Components related to movement artefacts were selected based on their spectral properties, and their spatial and temporal kurtosis, similar to Nolan et al. (2010). Note that only components that contributed to more than 5 % of the total data variance were rejected, as noisy components were expected to contribute largely to the total variance. We then reconstructed the data in sensor space by recombining the remaining components with their respective weights, as estimated by ICA. Forth, visual inspection served to reject the remaining noisy epochs if necessary. A different number of epochs was left per participant per condition, of which the minimum number available across conditions

per participant was selected for further analysis (see Fig. S2). Preserving the same number of epochs across conditions (although different between subjects) ensured that no artificial differences between conditions were added. Based on these pre-processing steps, four participants were rejected for further analysis due to excessive artefacts. This and subsequent analyses were conducted using the Fieldtrip toolbox (Oostenveld et al., 2011) with tailored functions.

Source analysis

We used exact low-resolution tomography (eLORETA) to estimate sources and cluster-based permutation to estimate their statistical significance.

Preparation of individual head models

In preparation for source estimation, individual head models were obtained from the MRIs using the SIMBIO implementation in Fieldtrip (Buchner et al., 1997). We used a 5-tissue finite element model (FEM) including the grey and white matter, cerebrospinal fluid (CSF), skull and scalp. These individual MRIs were segmented in SPM8. The resulting models were aligned to the electrodes' positions using the nasion and left and right auricular points. A 3D template grid with spacing of 5 mm in Montreal Neurological Institute (MNI) space was constructed based on the tissue probability map of the grey matter from the IXI dataset (http://brain-development.org/). This template grid was warped to each individual segmented grey tissue (Darvas et al., 2006; Evans et al., 2012; Brinkman et al., 2014), which allowed for a direct statistical comparison of grid-points among participants. Conductivity values of 0.33, 0.14, 1.79, 0.01 and 0.43 S/m were assigned to the grey matter, white matter, CSF, skull and scalp, respectively (Gabriel et al., 1996; Birot et al., 2014). The lead field, which defines the electrical field across tissues for every grid-point, was determined using the individual's FEM head model, electrodes' position and warped grid.

Source estimation

Exact LORETA (Pascual-Marqui, 2007) is a linear inverse solution that is considered robust to measurement and biological noise, as it assumes sources to contain neighbouring dipoles, i.e. a 3D array of grid-points, that exhibit similar current densities. We used the Fieldtrip's implementation of eLORETA which calculates spatial filters for every grid-point using the lead field and the cross-spectral density matrix of all the electrodes' combinations (Tan et al., 2015). Given the expected changes in the motor networks, we restricted analysis to the beta frequency band at 15-25 Hz. We note that modulations of this frequency band are associated with both unimanual (Pfurtscheller & Neuper, 1994; van Wijk et al., 2012a; Babiloni et al., 2016) and bimanual movements (Serrien & Brown, 2002; Gross et al., 2005; Boonstra et al., 2007; Houweling et al., 2010). Sources were estimated for all movement conditions and rest.

Statistics

Two statistical analyses were performed at source level. The first one contrasted *rest* with *task* to test if our procedures provided reliable neural sources. The second one contrasted all movement conditions (*task*) with each other to obtain sources that showed the main effect of frequency ratio. For both cases, we applied a cluster-based permutation test to estimate significance. Unlike parametric statistics, this test does not impose any assumptions of the probability distribution of the data (Maris & Oostenveld, 2007). In brief, cluster-based permutation consists of randomly partitioning the data and calculating a test statistic between the permuted datasets in two steps several times. First, we determined a statistic measure (a t-value or an F-value, depending on the effect of interest), and clustered significant grid-points based on spatial adjacency, i.e. grid-points located next to each other. Second, we computed a summary test on the clusters defined by both the intensity and the size of the cluster, i.e. the amplitude of the statistic and the number of grid-points, respectively. This summary test combining both parameters of the cluster is the weighted cluster mass which is considered sensitive to both high intensity and spatially extended signals (Bullmore *et al.*, 1999; Hayasaka & Nichols, 2004; Maris & Oostenveld, 2007).

After permuting the data 5000 times, we obtained a Monte-Carlo probability distribution based on the number of permutations with summary tests larger than the statistics obtained from the observed data, i.e. not permuted. For investigating the effect of the three conditions (*task*) on the source data, we used a dependent (paired) F-statistic and we defined the significance level at 0.05. By this, significant voxels indicated where the effect of frequency ratio was found, but note that no information of the direction of the effect is obtained. We selected a lower significance value (0.01) for the *rest* vs. *task* contrast because the neural involvement in both states can be expected to greatly differ across the whole brain; a higher significance value would render the resulting map meaningless. For this contrast, we used a one-sided dependent (paired) t-statistic.

Phase-based connectivity

The weighted phase-lag index (WPLI) is a reliable connectivity measure robust to volume conduction (Vinck *et al.*, 2011; Hardmeier *et al.*, 2014). Therefore, we assessed the functional connectivity among brain regions using the WPLI which estimates the leading or lagging node in a pair of signals weighted by the imaginary part of coherency (Vinck *et al.*, 2011). Also, in order to further reduce the effects of volume conduction and to have unbiased estimates of functional interactions between brain regions, the connectivity analysis was conducted at the source level (Srinivasan *et al.*, 1998). Regions of interest (ROIs) were selected based on the statistical map in the previous step (see below) and the time-series of the ROIs were estimated. The network modulations formed by the ROIs were evaluated with PLS analysis.

Phase synchronization

Based on the statistical F-map, we manually selected starting points around which a sphere of 2-cm radius was scanned to look for the grid-point with the maximum F-value. This grid-point was selected as an ROI. The F- and t-statistics of each ROI and their corresponding effect sizes were checked for final selection. Time-series of the specific coordinates of the ROIs were reconstructed using the spatial filters obtained from eLORETA estimation. Since the filter contains x-, y- and z-components, three constructed time-series were obtained per equivalent current dipole source. We applied singular value decomposition (SVD) to obtain the time-series accounting for most of the variance of the current dipole source regardless of the direction (Darvas *et al.*, 2004).

The ROIs' time-series were used to calculate the connectivity, i.e. the WPLI, between pairs of ROIs. The WPLI was calculated from the cross-spectral density of two ROIs at the frequency of interest (15-25 Hz), X, as follows:

$$WPLI = \frac{|E\{|Im\{X\}| * sign(Im\{X\})\}|}{E\{|Im\{X\}|\}}$$

where $E\{\cdot\}$ denotes the expectancy value; $Im\{\cdot\}$, the imaginary part of a complex value; and $sign(\cdot)$, the sign function (Vinck *et al.*, 2011). Connectivity measures were estimated for all the possible pairs of the selected ROIs to create an all-to-all network and averaged across trials per participant per condition.

Statistics

We applied partial least squares (PLS) analysis (Bookstein, 1994; McIntosh & Lobaugh, 2004; McIntosh & Mišić, 2013) to statistically test the modulations of the network as a whole. Two types of PLS analysis were performed: a mean-centring PLS to investigate the effects of our experimental design on the phase synchronization, and a behavioural PLS to look at the correlations between the phase synchronization and behaviour. For the mean-centring PLS, an average of the WPLI data across participants per condition per pair was calculated, after which the resulting matrix was mean-centred per pair, and the SVD was applied. In the behavioural PLS, the correlation matrix between the connectivity data and the behavioural data was calculated and SVD was applied. SVD served to detect latent variables (LV) that explained the mean-centred data, or the correlation between brain and behaviour, as much as possible, and had a "score" in the form of a singular value. Subsequently, we applied a permutation test to evaluate the significance of each LV. The data was permuted 5000 times, and for each time a PLS analysis was realised (McIntosh & Lobaugh, 2004). Akin to the permutation test described for source statistics, probability values were calculated as the ratio between: a) the number of times an LVs singular value obtained from the *original data* resulted in an equal or lower value than the LVs singular value obtained from the *permuted data*; and b) the total number of permutations. The significance level was set to $\alpha=0.05$.

For the significant LVs we tested stability of the mean-centred data and the correlations with behaviour using bootstrapped estimates of standard errors (the bootstrapping was repeated 5000 times). We determined the brain scores (brain saliences) by projecting the LV to the connectivity data. These saliences served as a summary measure per participant showing how strongly the network formed by the LV is present in each participant. To identify the stable connectivity pairs, bootstrapping ratios were calculated as the ratio of the salience value of each connectivity pair and the standard error of that pair from the resamples. We considered a pair stable when the bootstrap-derived ratio > 3 (Mentis et al., 2003; McIntosh & Lobaugh, 2004). We conducted two behavioural PLS tests: one with the participant-target deviation and one with the frequency-ratio deviation separately, because we expected to find different connectivity pairs involved in the performance of the visuomotor task and in the accuracy of the movement pattern. The stable pairs resulting from the mean PLS were selected to test whether the power in the nodes was driving the connectivity differences between the pairs (see Table S1 and Fig. S4).

Results

Behavioural results

There was a significant main effect of task complexity on the cursor-target deviation, $F(2, 50) = 71.894$, $p<0.001$. Post-hoc paired t-tests revealed significant differences across conditions ($p<0.001$ for all comparisons): errors increased with task difficulty (see Fig. 2). The corresponding means and standard error of the mean (SEM) of the cursor-target deviation were (in arbitrary units): 0.7184 (0.041) for the 1:1 ratio; 0.9342 (0.042) for the 1:3 ratio; and 1.0799 (0.043) for the 3:1 ratio.

Similar to the cursor-target deviation, we found a significant main effect of task difficulty on the frequency-ratio deviation, $F(2, 50) = 48.209$, $p<0.001$. Pairwise comparisons were significant in the post-hoc paired t-tests ($p<0.05$) with means and SEM of the frequency-ratio deviation (in degrees) of 0.945 (0.019) for the 1:1 ratio, 0.997 (0.022) for the 1:3 ratio, and 1.18 (0.036) for the 3:1 ratio.

In view of these results obtained with both behavioural measures, the error in both measures increased from 1:1, 1:3 to 3:1.

Source results

As expected, the *rest* vs. *task* contrast showed that the bilateral S1/M1 regions were involved in the bimanual task (see Fig. S3). This, however, does not preclude other regions being involved, because a stringent significance level was selected to pinpoint the biggest differences; see Methods section. On the other hand, we found several ROIs where power levels in the beta band differed significantly across all bimanual conditions. This was primarily focused on the right hemisphere (see Fig. 3). The effect of frequency ratio on the spectral power was significant in the right pre/post-central areas (henceforth called S1/M1, inferior and posterior), the right PMC, and the right precuneus.

Connectivity results

From the F- statistical map, four ROIs in the right hemisphere and their mirrored coordinates in the left hemisphere were selected for further analysis. As seen by the *rest* vs. *task* contrast and in agreement with the literature reviewed in the Introduction section, we expected prominent bilateral activation in the brain during a bimanual task. In order to test whether the selected ROIs were active during *task* with respect to *rest*, we evaluated their corresponding t-values and effect sizes (see Table 1). From this, we decided to retain seven ROIs and to reject the left PMC, given its negative t-value. The MNI coordinates and the corresponding F-statistic are listed in Table 1. The network comprised of 21 WPLI values per participant (22) and condition (3), i.e. $21 \times 22 \times 3$ values. Note that we excluded self-connections and that WPLI is commutative by which every network has 21 possibly independent connections. The averaged network for the 1:1 condition is shown in Fig. 4, and the averaged values per condition per pair are shown in Fig. 5.

The mean PLS revealed a significant 1st LV ($p<0.001$) with a singular value that accounted for 66.86 % of the total variance of the data. The task salience revealed a positive trend of the LV with task difficulty (see Fig. 6.a). The stable pairs with a bootstrap-derived ratio > 3 contained a link between right inferior S1/M1 and right precuneus (inter-ROI distance: 9.49 cm); right inferior S1/M1 and left precuneus (inter-ROI distance: 10.68 cm), and right superior S1/M1 and right PMC (inter-ROI distance: 5.61 cm).

The behavioural PLS with the *frequency-ratio deviation* as metric showed no significant LVs ($p=0.21$). The behavioural PLS with the *cursor-target deviation* as metric unravelled a significant 1st LV ($p=0.002$) that accounted for 47.92 % of the correlation between the connectivity data and the coordination measure. The correlations between the brain saliences of the 1st LV and the cursor-target deviation were positive, significant and stable (95 % confidence interval that does not cross zero) (see Fig. 6.b, and Fig. S5). The stable pairs contained a link between right superior S1/M1 and left inferior S1/M1 (inter-ROI distance: 10.5 cm).

Discussion

We investigated the neural correlates of bimanual movements with different difficulty levels, resulting in the identification of a neural network and correlation of its properties with behaviour. From our review and understanding of the current published literature, this is the first time that this question has been addressed using EEG connectivity measures from source-estimated time-series. As expected, non-isofrequency movements were performed less successfully than the isofrequency movement. When the non-dominant hand was assigned the faster cycling frequency (3:1), performance errors were larger than in the converse arrangement (1:3). Brain regions involved in the task with respect to rest included the frontal, and parietal regions, and those showing modulation with task difficulty included the right S1/M1, right PMC and right precuneus. We selected seven ROIs and found increases in beta synchronization associated with increased task difficulty and a positive correlation between

the connectivity of these regions and the performance error (cursor-target deviation). Specifically, the connectivity pairs between bilateral S1/M1s showed a stronger correlation than the other pairs with the cursor-target deviation. It should be noted that more accurate methods, such as the ones applied here, for estimation of time-series at the source level using M/EEG are not frequently used in the neuroscience field, and have not been applied before to investigate how the brain copes with motor task difficulty. Moreover, the use of realistic head models with high-density EEG to answer this question is innovative and increases confidence in the connectivity-related results.

Task allocation assignment affects bimanual performance

As hypothesised, there was a deterioration of performance as a function of the level of task difficulty: the lowest error scores were obtained during performance of the 1:1 ratio, followed by the 1:3 ratio and finally the 3:1 ratio. This effect was found with respect to both behavioural error measures. Our results are in line with previous studies reporting more accuracy and/or stability during isofrequency movements than non-isofrequency movements (e.g. deGuzman & Kelso, 1991; Swinnen *et al.*, 1997; Sisti *et al.*, 2011). Inter-hemispheric crosstalk through the corpus callosum may account for this difference (Marteniuk *et al.*, 1984; Swinnen *et al.*, 1992; Swinnen, 2002; Swinnen & Wenderoth, 2004; Daffertshofer *et al.*, 2005; Houweling *et al.*, 2010). Our design challenged the typical (preferred) task distribution between the left and right hand as a faster tempo was required for the non-dominant hand in the 3:1 condition, exerting a change of focus of attention from the dominant to the non-dominant hand, which led to increased performance difficulties. Deterioration of performance as a result of a less preferred compared to a preferred task allocation assignment may be an indication of the asymmetrical coupling between hands (Peters, 1985; Walter & Swinnen, 1990; Byblow *et al.*, 1998; Kourtis *et al.*, 2014). Interestingly, such asymmetrical performance is less common in highly skilled participants (Summers *et al.*, 1993; Peper *et al.*, 1995; Pressing *et al.*, 1996), suggesting that the preference towards isofrequency movements and assignment of the more complex subtask to the dominant hand in non-isofrequency movements, can be overcome by training.

Non-dominant hemisphere is modulated with task difficulty

As expected, we found pronounced contributions of bilateral sensorimotor cortices during the movement conditions compared to rest. Contrary to our hypothesis, however, only sensorimotor regions in the non-dominant hemisphere showed a modulation of beta power as a function of task difficulty. Our hypothesis was based on previous studies that have primarily addressed in- and anti-phase movements.

We would like to note that there is no current consensus regarding the presence of bilateral activation as a function of task difficulty in bimanual movements – see Rueda-Delgado *et al.* (2014) for a related review. Our results are in line with Houweling *et al.* (2008) who reported increased beta activity in the non-dominant hemisphere during a visually-guided non-isofrequency bimanual movement, and with Gross *et al.* (2005) who reported power

modulations in the non-dominant M1 with tasks starting from unimanual, to in-phase and to anti-phase movements. The lack of consensus may be a result of the type of movement executed. In the majority of the studies listed in the Introduction, bimanual movements were contrasted to unimanual movements. Only one study investigated the difference between non-isofrequency and isofrequency movements using fMRI, and, similar to our results, did not find involvement of the left S1/M1, but did find involvement of the right S1/M1 (Ullén *et al.*, 2003). In our study, we included different frequency ratios for the rotational movement, increasing the spatiotemporal demands of the task with the assistance of visual feedback, which could gear visual attention towards monitoring of the more vulnerable non-dominant hand. Visual attention (Corbetta *et al.*, 1993), visuomotor tracking (Brown *et al.*, 2004), error detection and directional interference in bimanual movements (Wenderoth *et al.*, 2004), and guidance with augmented visual feedback in bimanual movements (Beets *et al.*, 2015) have underscored an important role for right hemisphere regions (Serrien *et al.*, 2006). A combination of these functions is also required in the visually-guided task used here.

The modulations of power in the right hemisphere as a function of task difficulty might be a consequence of deployment of attention to the weaker non-dominant hand for which there is less optimal control. We contend that the left hemisphere exerts primary control over movements, including bimanual movement. However, when an unskilled performer is required to produce new complex movements, it is likely that more neural resources in the right hemisphere are recruited to monitor the non-dominant hand until a sufficient performance level is obtained via dedicated training.

The neural network composition associated with bimanual coordination extends beyond the basic motor areas including other areas, depending on task context. Imaging studies suggest that the bilateral precunei are involved in encoding motor goals (Bernier & Grafton, 2010), in the spatial location of environmental features over time (Galati *et al.*, 2010), and in coordination efforts as required when both hands perform movements with different spatiotemporal features simultaneously (Wenderoth *et al.*, 2005). This is a specific feature of our task, because participants received augmented visual online feedback (external) during every trial and this augmented sensory input was processed in association with incoming proprioceptive information (internal) during execution.

Long-range synchronization is modulated with task difficulty and correlated with increased performance error

Long-range synchronization across ROIs, measured with phase-based connectivity, was modulated with task difficulty and was correlated to behaviour during execution of the bimanual visuomotor task. This is consistent with previous studies across many domains indicating large-scale cortical synchronization accompanying sensorimotor and multisensory integration (e.g. Roelfsema *et al.*, 1997; Maier *et al.*, 2008), perception (e.g. Hipp *et al.*, 2011), selective attention (e.g. Gregoriou *et al.*, 2009), decision making (e.g. Pesaran *et al.*, 2008), working memory (e.g. Palva *et al.*, 2010), and movement production (van Wijk *et al.*, 2012b).

We found that the performer exhibits tighter interaction between brain areas, as evidenced by increased large-scale synchronization with increasing task demands. As stated in the Introduction, previous studies have reported increases of connectivity with increasing task demands in bimanual movements (Gerloff & Andres, 2002; Serrien & Brown, 2002; Serrien *et al.*, 2003; Rissman *et al.*, 2004; Sun *et al.*, 2004; Maki *et al.*, 2008; Walsh *et al.*, 2008; Houweling *et al.*, 2010; Heitger *et al.*, 2013); but also in unimanual movements (Andres *et al.*, 1999; Babiloni *et al.*, 2011; Volz *et al.*, 2015), and cognitive tasks (e.g. Burianová *et al.*, 2013; Luft, 2014). The aforementioned studies primarily focused on connectivity between bilateral S1/M1s and SMA and on the preferred in-phase/anti-phase coordination modes. Hence we extended the investigation to a 7-ROI network including parietal areas during bimanual movements of different spatiotemporal features. Particularly, our findings support those of Heitger *et al.* (2013) who reported increased fMRI connectivity in anti-phase compared to in-phase movements using graph theoretical network analysis. Finding the same effect with a broader network and with manipulations of the spatiotemporal complexity of the bimanual coordination pattern indicates that there is a more generic long-range dynamic reorganization in response to higher task demands that is neither restricted to motor areas nor to the preferred coordination modes. Although not manipulating task difficulty, previous studies reported increased functional connectivity in task-related and resting-state functional networks in older compared to young adults (Heitger *et al.*, 2013; Solesio-Jofre *et al.*, 2014), and after a training scheme in young (Heitger *et al.*, 2012) and older adults (Klados *et al.*, 2016). Thus, changes in functional connectivity at the short-term (related to task difficulty or motor learning) and the long-term (along the lifespan) seem to be critical for better understanding behaviour.

Specifically, the stable pairs that showed the most prominent modulation with task difficulty were the links between the right S1/M1 and the bilateral precuneus, and between the right S1/M1 and right PMC. The precuneus-right S1/M1 pair might reflect the prominent visuomotor control feature of the task, and the attentional deployment towards more difficult movements. As mentioned above, the precuneus is involved in visuomotor tracking (Brown *et al.*, 2004), but also in pursuit movements and saccades (Berman *et al.*, 1999), attention orientation (Simon *et al.*, 2002), visual tracking of moving targets (Culham *et al.*, 1998), and complex bimanual movements (Wenderoth *et al.*, 2005); for a review see Cavanna and Trimble (2006). Synchronization between medial-parietal and sensorimotor cortices might be crucial for multisensory integration, though the detailed mechanism remains unknown. Modelling work suggests that multisensory integration relies on long-range synchronization across frequencies, as modelled with beta-gamma coupling in the motor and visual cortices during motor imagery (van Wijk *et al.*, 2013), and simulated with delta-alpha coupling in resting state (Jirsa & Müller, 2013). Conversely, the link between right S1/M1 and right PMC might reflect increased intra-hemispheric inhibition to reduce left/right interference as proposed by the model of Daffertshofer *et al.* (2005).

In addition to connectivity modulations with task difficulty, we also aimed for brain-behaviour correlations as these reflect the relationship between brain states and the associated

product of that state, i.e. behaviour, and not the relationship between brain states and arbitrary task definitions (Sidtis, 2012). The behavioural results showed increased errors across the different coordination modes and, hence, performance error and task difficulty were intrinsically related. This was confirmed by the two types of analyses performed: mean PLS which showed modulations of beta synchronization as a function of task difficulty, and behavioural PLS which showed correlations of beta synchronization with performance error. Furthermore, task saliences of mean PLS reflected the change of task allocation from the isofrequency to non-isofrequency movements. Specifically, the positive correlations of synchronization with error hint at the role of synchronization as information carrier of motor performance and/or control in the brain (Fries, 2005; van Wijk *et al.*, 2012b). This finding supports the notion that changes in task-related functional connectivity mediate behavioural performance, which was previously reported in cognitive tasks (McIntosh *et al.*, 2003; Lewis *et al.*, 2009), and it expands the notion to bimanual tasks. Similar studies focused on network reorganization occurring during motor learning (Serrien & Brown, 2003; Mehrkanoon *et al.*, 2016). However, to the best of our knowledge, this is the first report of connectivity-behaviour correlations during bimanual non-isofrequency movements. Using fMRI, Heitger *et al.* (2013) found no association between behavioural measures of in- and anti-phase movements and functional connectivity in young participants. There might be at least two reasons for this difference. First, the modulations of functional connectivity with behaviour might be more prevalent with bimanual tasks diverging from the preferred stable modes. Second, the underlying mechanism sustaining connectivity in fMRI data is inherently different from the analogous mechanism in EEG data.

The positive correlation with the performance error (*cursor-target* deviation) revealed the pair comprised by the bilateral S1/M1s to be more stable. The bilateral S1/M1 pair confirms previous evidence of the important role of modulation of inter-hemispheric connectivity during execution of various bimanual movements (Gerloff *et al.*, 1998; Gerloff & Andres, 2002; Rissman *et al.*, 2004; Stinear & Byblow, 2004; Swinnen & Wenderoth, 2004; Houweling *et al.*, 2008; Maki *et al.*, 2008; Houweling *et al.*, 2010).

Study limitations

Source localisation for (sub)cortical structures is a challenge because the electrical signal is attenuated through different tissues before it is measured as scalp EEG. Furthermore, volume conduction may severely affect the EEG, which may yield a strong, confounding bias when it comes to the functional connectivity between estimated sources. We reduced this potential pitfall by: a) conducting connectivity analyses of source-level neural activity reconstructed using a realistic head model; and b) using connectivity measures that are robust against volume conduction (van Wijk *et al.*, 2012b). Additionally, temporal modulations of neural activity occurring at short time scales (ms range) were not considered in this study. The smooth continuous rotation of the wrist employed here rendered it difficult to define anchor points in the train of movement. Our results therefore demonstrate overall changes in the beta band across the entire movement.

Conclusion

The scientific community has become increasingly interested in investigating how neural populations interact with each other at small and larger time scales and how these interactions are modulated as a function of task demands and/or levels of behaviour (or error). In this study, we used: a) high-density EEG to investigate neural dynamics in the beta band; b) individual head models and finite element modelling for source estimation; and c) multivariate statistics for assessing modulations in neural connectivity during the execution of a bimanual task. We found that increases of distant cortical synchronization in the beta band are a mechanism for coping with increasing task demands in a bimanual visuomotor movement with different spatiotemporal features. This interpretation finds support by clear correlations between synchronization measures and performance errors. Our findings extend previous work of neural synchronization during tasks requiring cognitive function and sensorimotor integration.

Our study pertains to bimanual execution after a short practice session. For a more comprehensive view of bimanual motor execution, cortical reorganization occurring during other stages of motor learning will need to be investigated.

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Fig. 1 Participants rotated the dials in an outward orientation at different frequency ratios, indicated by the slope of the cue line (blue).

Fig. 2 A main effect of frequency ratio was found on the cursor-target deviation and the frequency-ratio deviation. Black circles indicate the mean and the error bars indicate the standard error of the mean.

Fig. 3 Inflated cortical surfaces with overlaying statistical map of the F-test contrasting all movement conditions. Seven ROIs were selected for the connectivity analysis. Voxels shown in colour were significantly different among movement conditions ($p<0.05$).

Fig. 4. Averaged connectivity matrix of condition 1:1 with 7 ROIs. WPLI is colour-coded.

Fig. 5. Averaged WPLI per condition per pair indicated by the number on top of the bar. Stable pairs resulting from the mean PLS are highlighted in black. Note that the y-axis has been adjusted in each bar graph to better visualize the differences across conditions.

Fig. 6. PLS results. a) Task saliences of Mean PLS show a modulation of connectivity with task difficulty. b) Pearson's correlations of brain saliences of 1st LV and the cursor-target deviation, separated by condition. Error bars represent bootstrapped 95% confidence intervals.

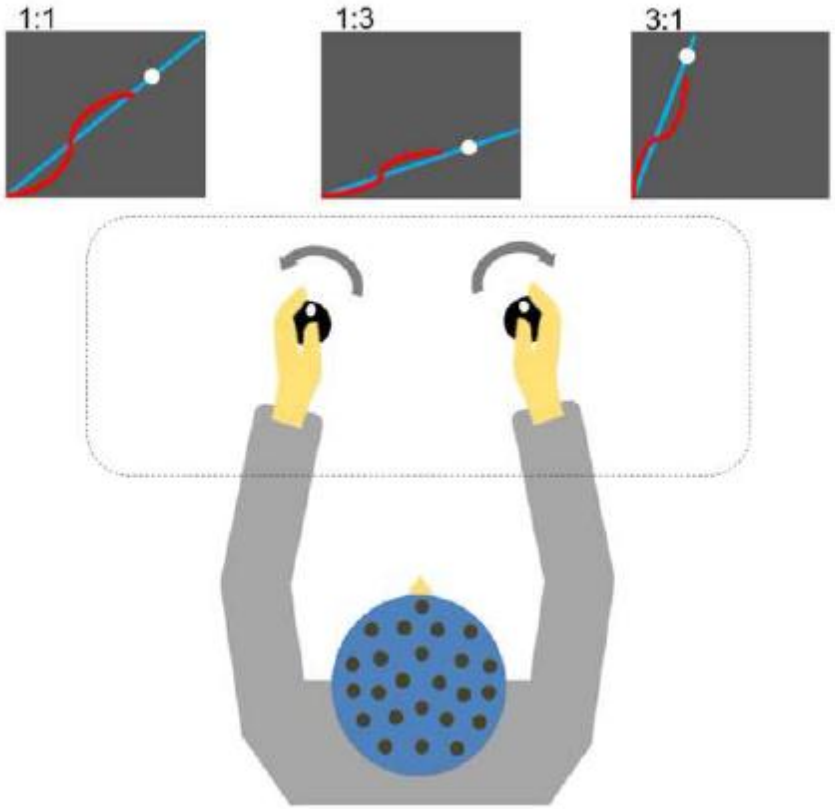
Table 1. MNI coordinates, statistics and corresponding effect sizes of ROIs. The F-statistics was calculated from the F-test including three movement conditions. The t-statistics was calculated from the paired samples t-test performed between *rest* and *task*. Left PM was not included in further analysis as its involvement with respect to *rest* could not be confirmed with this data (see Results section).

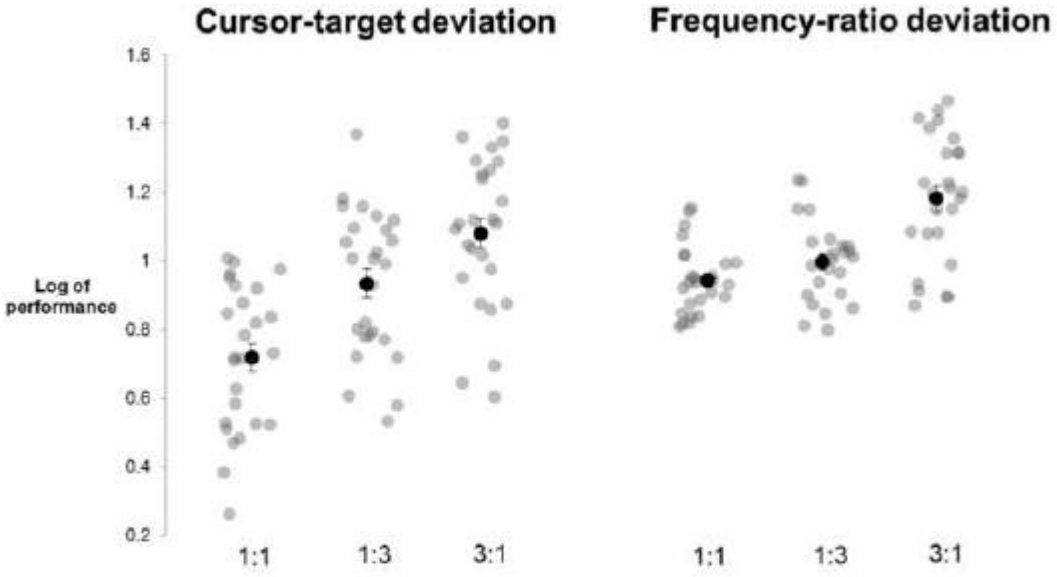
Regions' labels	MNI coordinates (mm)	F value	Eta	t-value	Cohen's d

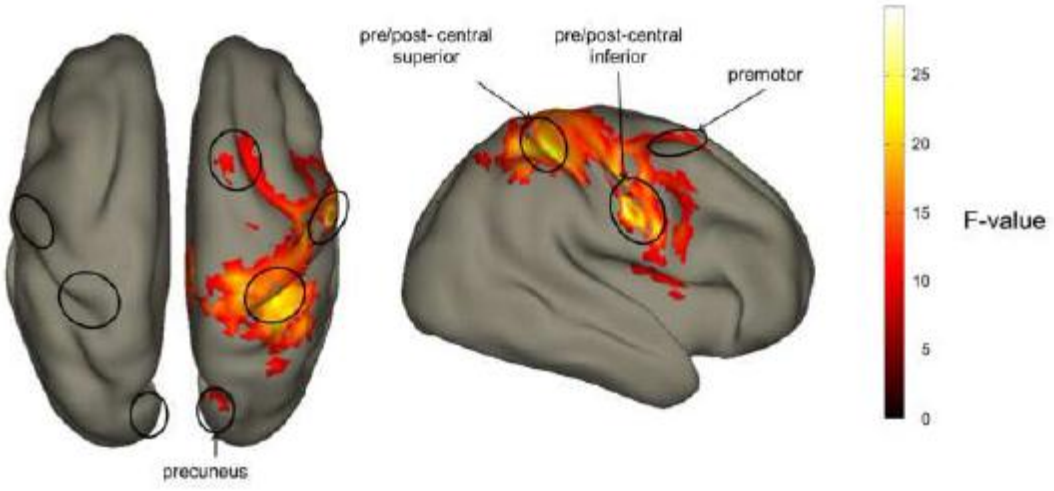
	x	y	z		squared		
R S1M1 inferior	60	-5	35	16.65	0.172	1.21	0.182
R S1M1 superior	35	-35	60	16.09	0.91	2.497	0.681
R PM	20	10	55	12.3	0.142	0.887	0.011
R precuneus	15	-80	35	11.89	0.039	2.933	0.329
L S1M1 inferior	-60	-5	35	2.739	0.061	0.68	0.130
L S1M1 superior	-35	-35	60	0.029	0.001	2.455	0.716
L PM	*-20*	*10*	*55*	*3.571*	*0.115*	*-0.148*	*0.116*
L precuneus	-15	-80	35	7.161	0.041	2.615	0.381

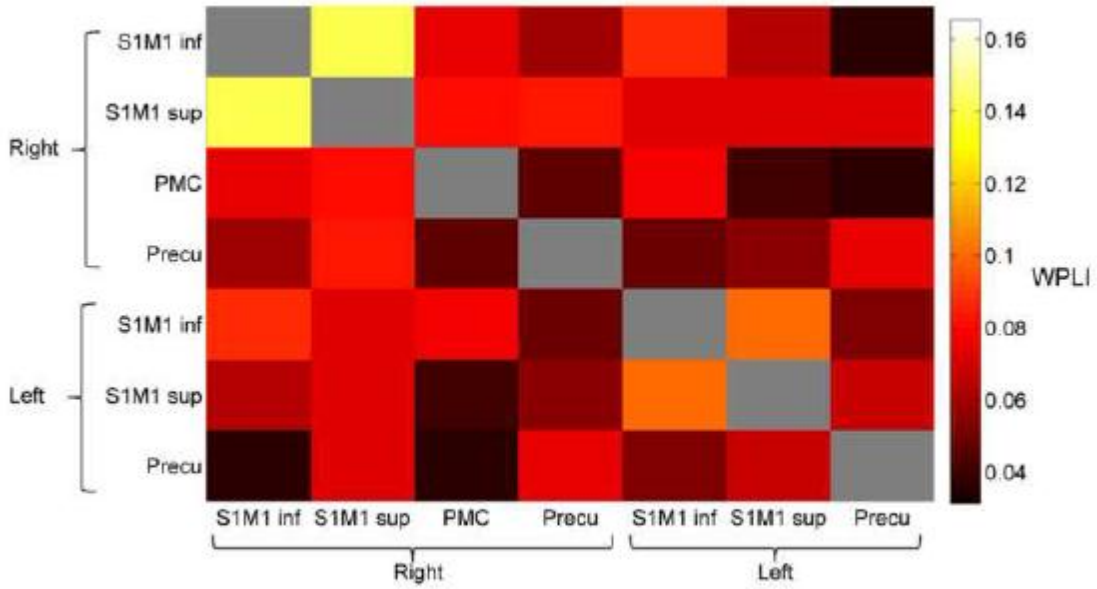
Highlights

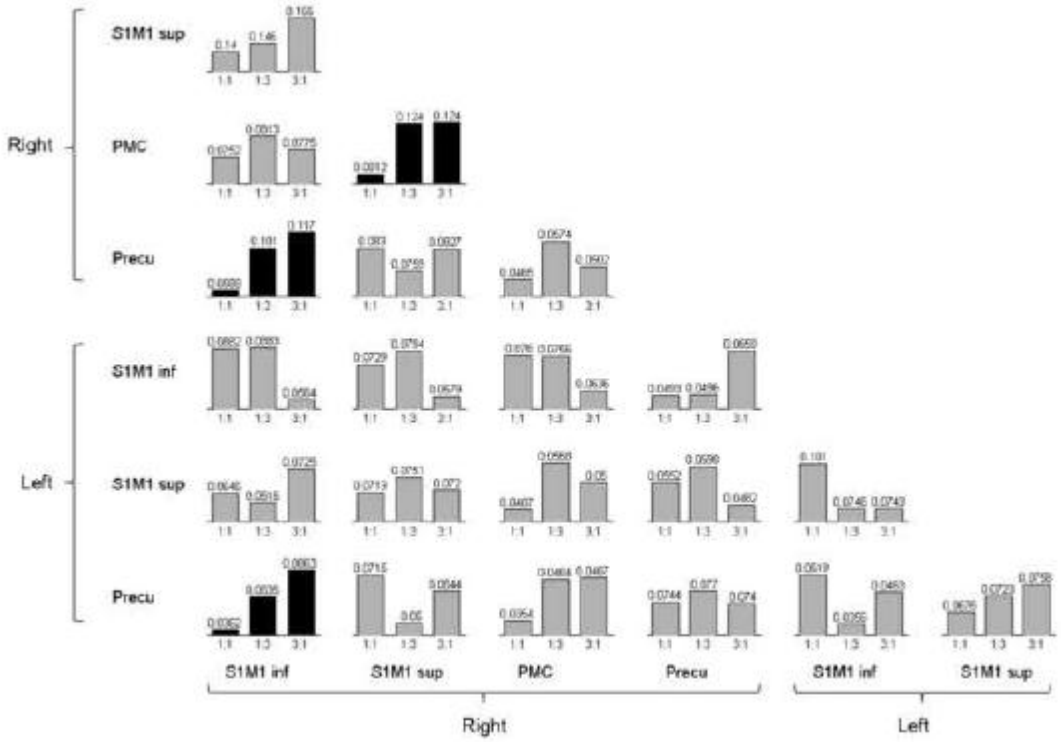
- Neural processes underlying bimanual coordination were investigated with high-density EEG.
- Beta power in non-dominant hemisphere is modulated with task difficulty.
- Inter-hemispheric beta coupling increases with task difficulty / error of performance.



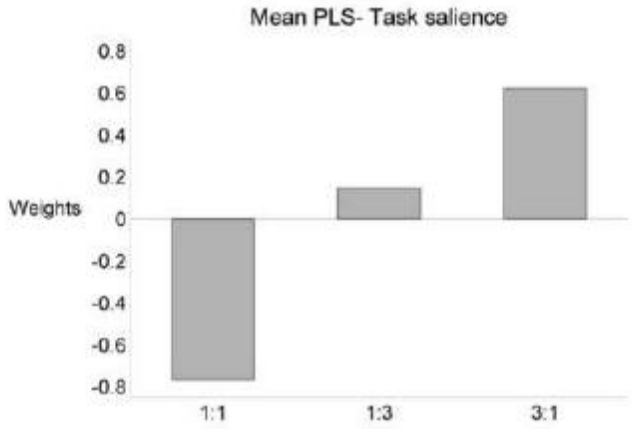








a)



b)